molecules

# Supplementary Material:

# Identification of Flavonoids as Putative ROS-1 Kinase Inhibitors using Pharmacophore Modeling for NSCLC therapeutics

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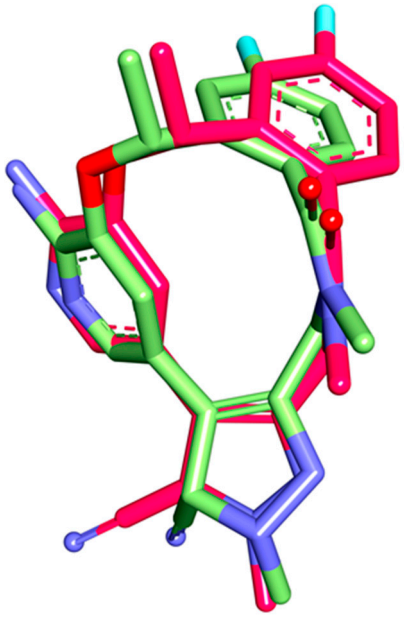

**Figure S1.** Overlay of the docked pose (green) of lorlatinib with its crystal structure conformation (pink) in PDB ID: 4UXL.

**Table S1.** The docking scores and intermolecular interactions of lorlatinib and flavonoids from *TimTec* database with ROS-1 wild-type (WT) tyrosine kinase domain.

| *TimTec* ID No. | Gold Score | Hydrogen Bond Interactions | Hydrophobic and van der Waals Interactions |
|---|---|---|---|
| ST4119644 | 72.80 | Lys1980, Met2029 | Leu1951, Val1959, Glu1961, Ala1978, Lys1980, Glu1997, Met2001, Leu2010, Leu2026, Leu2028, Glu2030, **Gly2032**, Leu2086, Gly2101, Asp2102, Gly2104 |
| ST051039 | 61.78 | Lys1980, Glu1997, Met2029 | Leu1951, Val1959, Ala1978, Met2001, Leu2010, Ile2024, Leu2026, Glu2027, **Gly2032**, Leu2086, Gly2101 |
| ST052370 | 52.40 | Met2029, Asp2033 | Leu1951, Val1959, Ala1978, Lys1980, Met2001, Leu2010, Leu2026, Glu2027, Leu2028, Glu2030, **Gly2032**, Leu2086, Gly2101, Asp2102 |
| ST077081 | 51.68 | Lys1980, Gly2101 | Leu1951, Val1959, Ala1978, Leu2010, Leu2026, Leu2028, Met2029, Glu2030, **Gly2032**, Leu2086, Asp2102 |
| ST063011 | 51.05 | Lys1980, Met2029 | Leu1951, Val1959, Ala1978, Glu1997, Met2001, Leu2010, Leu2026, Glu2027, Leu2028, Glu2030, **Gly2032**, Leu2086, Gly2101, Asp2102, Phe2103, Gly2104 |
| ST4146854 | 50.31 | Lys1980, Met2029 | Leu1951, Val1959, Ala1978, Glu1997, Leu2010, Leu2026, Glu2027, Glu2030, **Gly2032**, Leu2086, Gly2101 |
| ST50843253 | 49.01 | Met2029 | Leu1951, Gly1952, Ser1953, Gly1954, Val1959, Ala1978, Lys1980, Leu2010, Leu2026, Glu2027, Glu2030, Leu2028, **Gly2032**, Leu2086 |
| ST50837833 | 48.11 | Lys1980, Met2029 | Leu1951, Val1959, Glu1961, Ala1978, Leu2010, Leu2026, Glu2027, Glu2030, **Gly2032**, Leu2086, Gly2101, Asp2102 |
| ST50707205 | 47.07 | Lys1980 | Leu1951, Ser1953, Gly1954, Val1959, Ala1978, Met2001, Leu2010, Leu2026, Leu2028, Met2029, **Gly2032**, Arg2083, Asn2084, Leu2086, Gly2101, Asp2102, Phe2103 |
| ST096317 | 46.15 | Lys1980, Glu2027, Met2029 | Leu1951, Gly1952, Gly1954, Val1959, Ala1978, Leu2010, Leu2026, Leu2028, Glu2030, **Gly2032**, Leu2086 |
| Lorlatinib | 43.74 | Glu2027, Met2029, **Gly2032** | Leu1951, Gly1952, Gly1954, Val1959, Ala1978, Lys1980, Leu2010, Leu2026, Leu2028, Asp2033, Leu2086 |

**Table S2.** The docking scores and intermolecular interactions of lorlatinib and flavonoids from *TimTec* database with ROS-1 mutated (MT) tyrosine kinase domain.

| *TimTec* ID No. | Gold Score | Hydrogen Bond Interactions | Hydrophobic and van der Waals Interactions |
|---|---|---|---|
| ST4119644 | 73.62 | Lys1980, Met2029 | Leu1951, Gly1954, Val1959, Glu1961, Ala1978, Lys1980, Glu1997, Met2001, Leu2010, Leu2026, Glu2027, Leu2028, Glu2030, **Arg2032**, Leu2086, Gly2101, Asp2102, Phe2103, Gly2104 |
| ST051039 | 64.34 | Met2029, Gly2101 | Leu1951, Val1959, Ala1978, Lys1980, Leu2010, Leu2026, Leu2028, Glu2030, **Arg2032**, Leu2086 |
| ST50843253 | 56.37 | Met2029, **Arg2032** | Leu1951, Gly1952, Val1959, Ala1978, Leu2026, Glu2027, Leu2028, Met2029, Leu2010, Glu2030, **Arg2032**, Asp2033, Arg2083, Leu2086 |
| ST052370 | 55.49 | Met2029 | Leu1951, Gly1952, Val1959, Ala1978, Leu2026, Leu2028, Glu2030, **Arg2032**, Asp2033, Arg2083, Asn2084, Cys2085, Leu2086, Asp2102 |
| ST077081 | 53.62 | Lys1980, Gly2101 | Leu1951, Val1959, Ala1978, Leu2010, Leu2026, Leu2028, Met2029, **Arg2032**, Leu2086, Asp2102 |
| ST50837833 | 51.49 | Lys1980 | Leu1951, Val1959, Ala1978, Leu2026, Leu2028, Met2029, Glu2030, **Arg2032**, Leu2086 |
| ST50707205 | 51.45 | Lys1980 | Leu1951, Gly1952, Gly1954, Ser1953, Val1959, Ala1978, Leu2010, Leu2026, Leu2028, Met2029, Glu2030, **Arg2032**, Asp2033, Arg2083, Asn2084, Leu2086, Asp2102, Phe2103 |
| ST4146854 | 50.24 | Met2029, **Arg2032** | Leu1951, Gly1952, Val1959, Ala1978, Leu2028, Glu2030, Gly2031, **Arg2032**, Asp2033, Arg2083, Leu2086 |
| ST063011 | 50.1 | Lys1980, Met2029 | Leu1951, Gly1954, Ala1955, Val1959, Ala1978, Leu2010, Leu2026, Glu2027, Leu2028, Glu2030, **Arg2032**, Leu2086, Gly2101, Asp2102 |
| Lorlatinib | 49.37 | Glu2027, Met2029, **Arg2032** | Leu1951, Gly1952, Gly1954, Val1959, Ala1978, Lys1980, Leu2010, Leu2026, Leu2028, Asp2033, Leu2086, Asp2102 |
| ST096317 | 47.15 | Lys1980, Glu2027, Met2029 | Leu1951, Gly1952, Gly1954, Val1959, Ala1978, Lys1980, Leu2010, Leu2026, Leu2028, **Arg2032**, Leu2086 |

**Table S3.** Binding free energy scores of the identified flavonoids with wild-type (WT) ROS-1 kinase computed through MM/PBSA methodology.

| TimTec ID | van der Waals (kJ/mol) | Electrostatic (kJ/mol) | Polar solvation (kJ/mol) | SASA energy (kJ/mol) | Binding energy $\Delta G_{bind}$ (kJ/mol) |
|---|---|---|---|---|---|
| ST4119644 | -206.983+/-17.338 | -74.699+/-18.331 | 214.499+/-24.603 | -24.502+/-1.074 | -91.685+/-20.795 |
| ST50837833 | -156.498+/-9.550 | -2.332+/-4.885 | 87.358+/-12.881 | -17.287+/-0.860 | -88.759+/-16.448 |
| ST096317 | -124.560+/-8.289 | -22.887+/-5.553 | 72.266+/-8.211 | -13.562+/-0.770 | -88.744+/-9.773 |
| Lorlatinib | -182.556+/-10.399 | -30.294+/-6.600 | 142.724+/-12.717 | -18.117+/-0.778 | -88.244+/-12.933 |
| ST50707205 | -175.441+/-17.394 | -30.543+/-10.584 | 140.517+/-20.621 | -19.406+/-1.240 | -84.873+/-14.681 |
| ST052370 | -158.976+/-13.663 | -25.749+/-18.882 | 126.783+/-36.610 | -18.315+/-1.358 | -76.258+/-12.242 |
| ST4146854 | -152.033+/-12.001 | -35.504+/-8.556 | 133.149+/-30.010 | -17.238+/-1.140 | -71.625+/-20.226 |
| ST063011 | -140.414+/-14.603 | -16.669+/-15.958 | 103.442+/-25.036 | -16.511+/-1.377 | -70.151+/-10.935 |
| ST077081 | -126.006+/-11.536 | -34.127+/-14.307 | 117.876+/-16.187 | -15.954+/-0.952 | -58.211+/-11.421 |
| ST051039 | -158.842+/-9.316 | -71.393+/-27.799 | 191.585+/-31.463 | -19.234+/-0.794 | -57.884+/-15.203 |
| ST50843253 | -112.840+/-11.108 | -25.897+/-13.760 | 105.361+/-19.246 | -14.422+/-0.757 | -47.798+/-16.563 |

**Table S4.** Binding free energy scores of the identified flavonoids with mutated (MT) ROS-1 kinase computed through MM/PBSA methodology.

| TimTec ID | van der Waals (kJ/mol) | Electrostatic (kJ/mol) | Polar solvation (kJ/mol) | SASA energy (kJ/mol) | Binding energy $\Delta G_{bind}$ (kJ/mol) |
|---|---|---|---|---|---|
| ST4119644 | -225.602+/-12.106 | -14.952+/-13.683 | 163.374+/-20.851 | -25.854+/-1.088 | -103.035+/-16.223 |
| ST051039 | -163.533+/-11.452 | -100.917+/-8.289 | 204.277+/-12.685 | -20.357+/-1.228 | -80.531+/-12.060 |
| Lorlatinib | -171.545+/-11.137 | -26.433+/-9.173 | 140.720+/-16.636 | -18.246+/-1.187 | -75.505+/-12.053 |
| ST096317 | -141.478+/-8.771 | -9.587+/-8.261 | 93.500+/-14.323 | -15.599+/-0.751 | -73.165+/-11.518 |
| ST077081 | -150.416+/-10.061 | -50.242+/-14.394 | 148.162+/-14.466 | -16.474+/-0.731 | -68.970+/-12.966 |
| ST50837833 | -158.519+/-8.741 | -5.606+/-6.718 | 112.490+/-16.206 | -17.327+/-0.759 | -68.962+/-10.945 |
| ST052370 | -164.272+/-10.392 | 4.860+/-6.486 | 110.367+/-11.953 | -19.445+/-0.748 | -68.490+/-12.688 |
| ST50707205 | -192.144+/-11.731 | -38.256+/-11.542 | 187.196+/-22.847 | -20.629+/-1.010 | -63.832+/-17.966 |
| ST063011 | -150.507+/-7.692 | -23.358+/-7.686 | 128.758+/-14.294 | -17.195+/-0.791 | -62.302+/-11.448 |
| ST4146854 | -131.148+/-9.103 | -29.211+/-9.581 | 127.515+/-14.325 | -15.781+/-1.071 | -48.626+/-12.066 |
| ST50843253 | -143.703+/-17.785 | -1.242+/-10.677 | 115.513+/-41.590 | -16.707+/-1.560 | -46.139+/-22.786 |

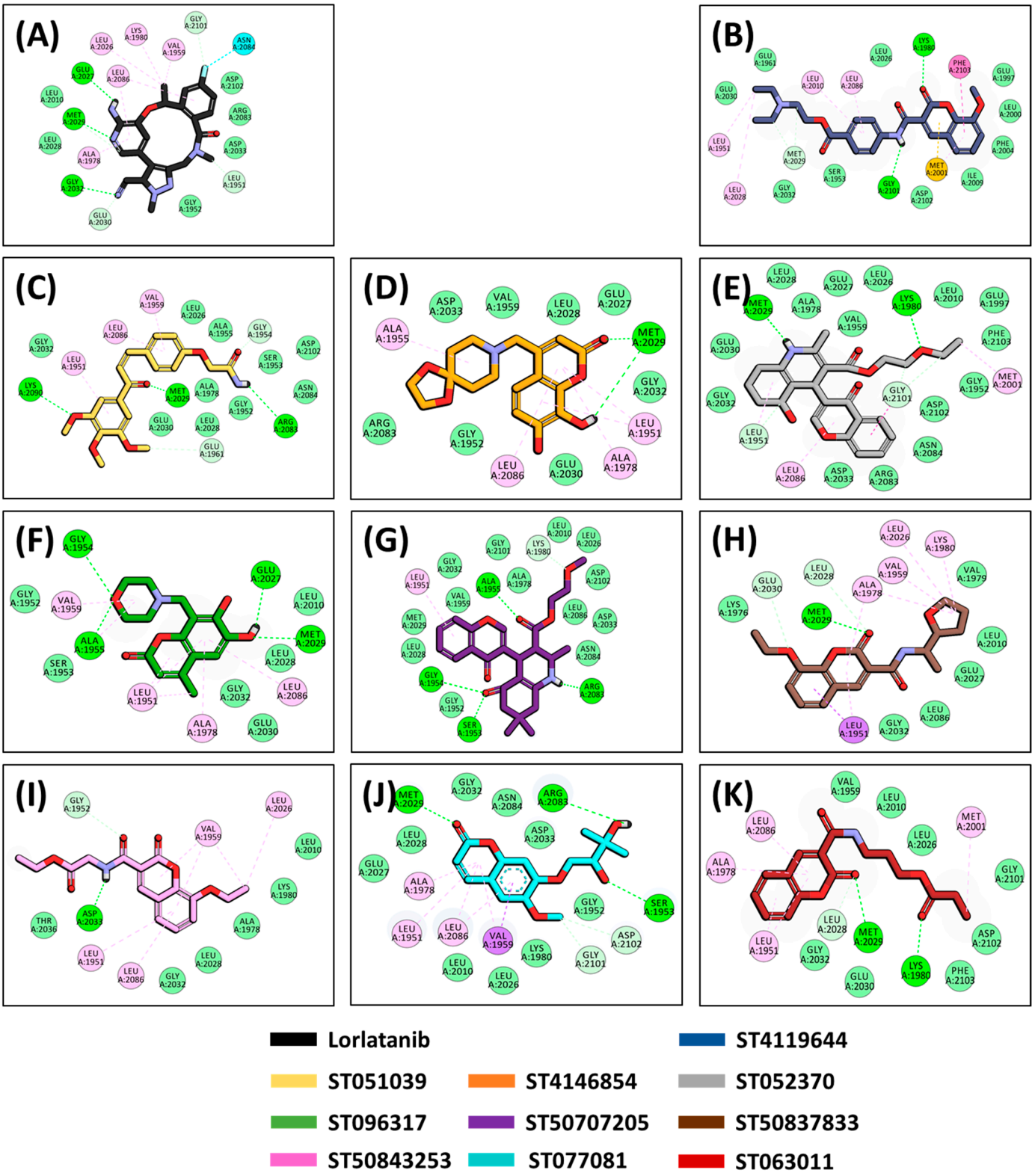


**Figure S2.** Binding interaction of flavonoids with key residues of the wild-type (WT) ROS-1 kinase domain. The flavonoids are represented as sticks. Hydrogen bonds are indicated as green dashed lines, hydrophobic interactions are shown as pink and purple spheres and the van der Waals interactions are displayed as light green spheres.

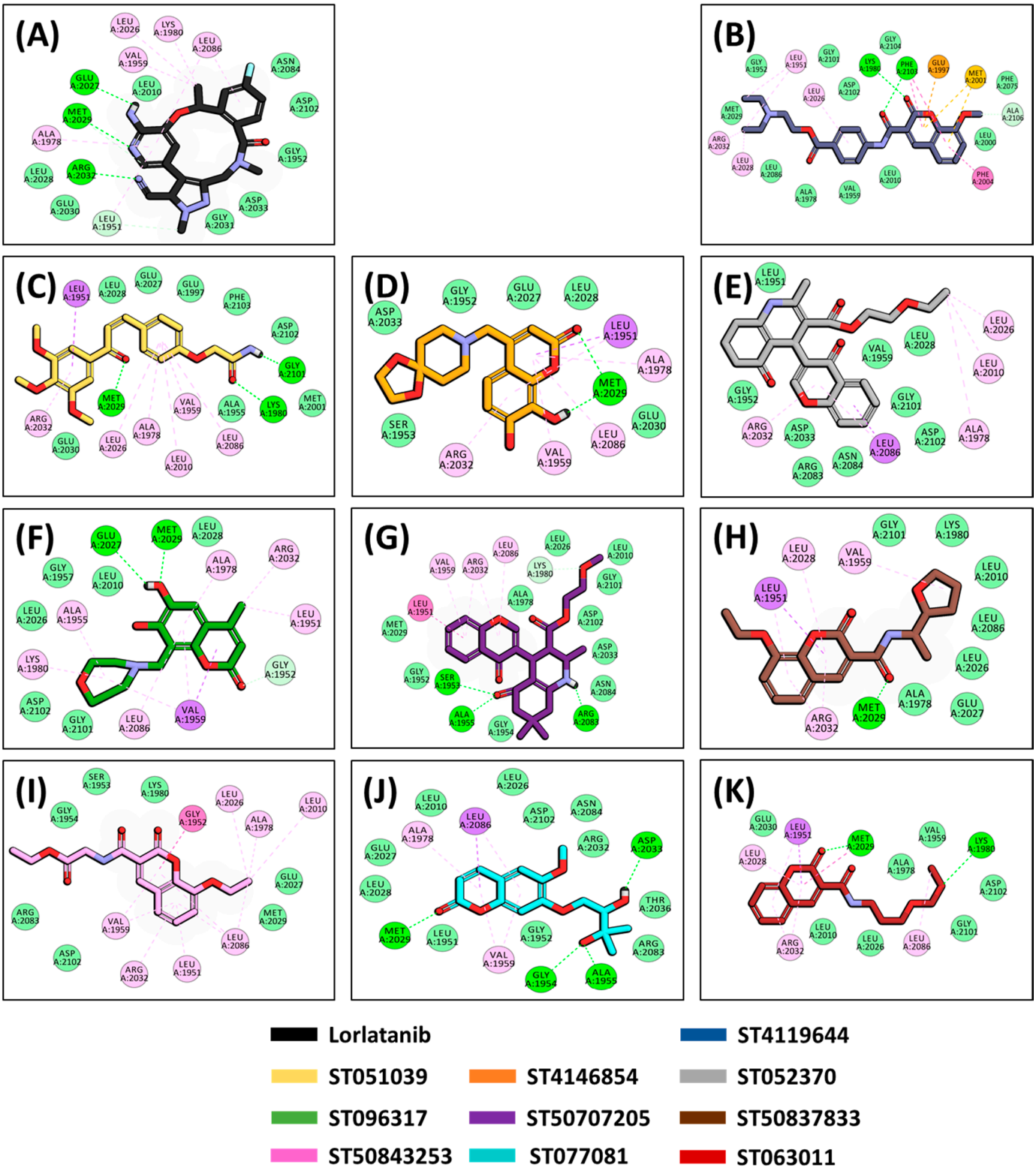


**Figure S3.** Binding interaction of flavonoids with key residues of the mutated (MT) ROS-1 kinase domain. The flavonoids are represented as sticks. Hydrogen bonds are indicated as green dashed lines, hydrophobic interactions are shown as pink, purple and orange spheres and the van der Waals interactions are displayed as light green spheres.

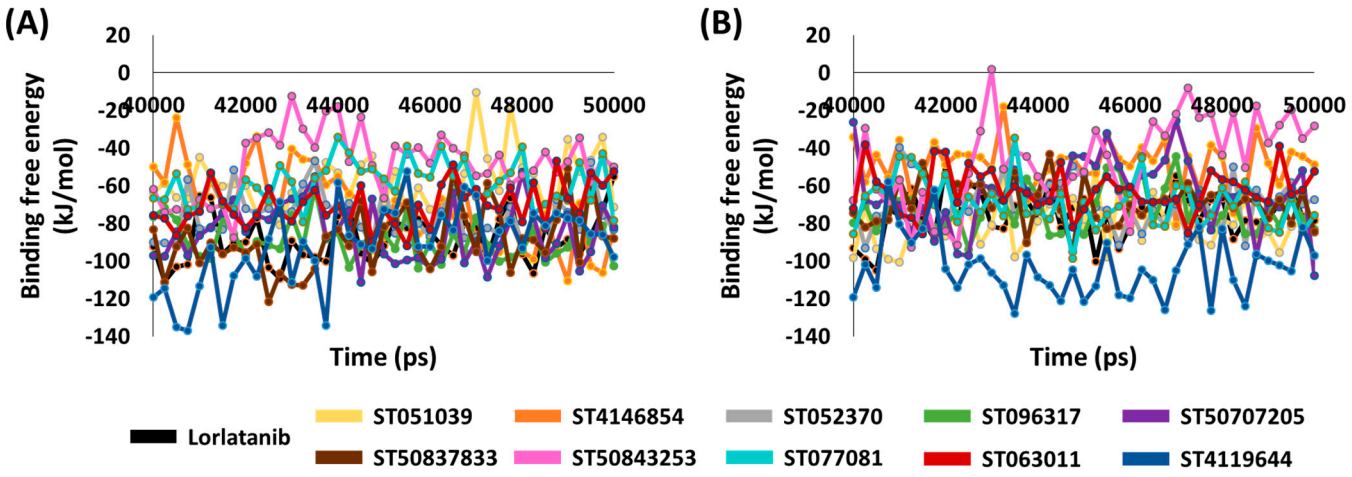


**Figure S4.** Binding free energy (BFE) analysis of (A) wild-type (WT) and (B) mutated (MT) ROS-1 systems.

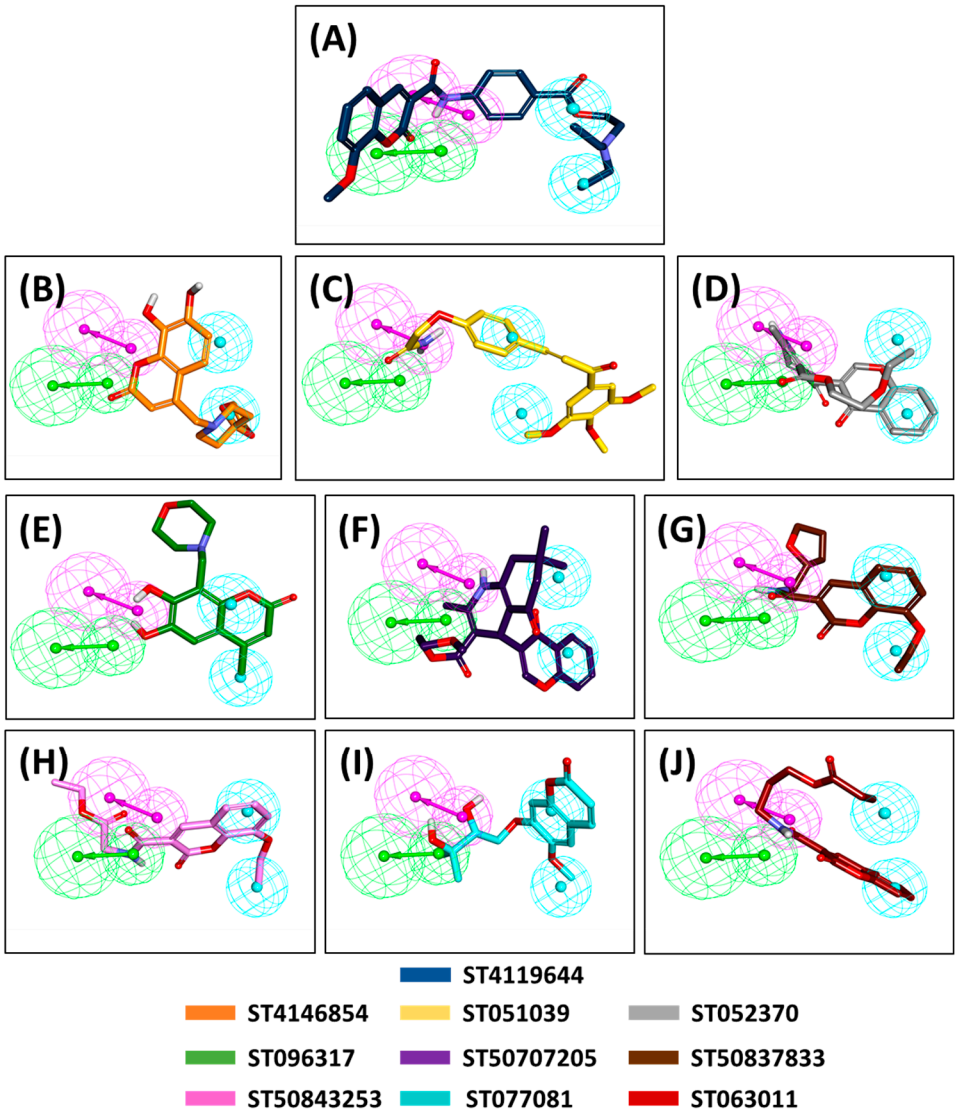


**Figure S5.** Alignment of the identified flavonoids with the pharmacophoric features. All flavonoids ((B) - (J)) including the (A) Hit compound represent the HBA (hydrogen bond acceptor), Hy (hydrophobic) and HBD (hydrogen bond donor) features of *Pharmacophore_01*.